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Efficiency of pyoverdines in iron removal from flocking asbestos waste: An innovative bacterial bioremediation strategy

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31 *P. mandelii*. Pyoverdine-asbestos weathering could therefore become an innovative method to
32 reduce anthropogenic waste.

33 **Keywords**

34 Pyoverdine- *Pseudomonas*-Asbestos-Iron-Alteration

35

36 **1. Introduction**

37 Asbestos has been extensively used because of its excellent properties, such as thermal
38 conductivity, high mechanical strength, resistance to chemical attacks, and low cost. Asbestos is
39 composed a group of fibrous silicate minerals that belong to the serpentine and amphibole
40 families. Amphiboles can contain up to 36% iron by weight, whereas the serpentine form of
41 asbestos contains only 2 to 3% iron by weight (Chao and Aust, 1994). Iron can be present as part
42 of the mineral structure in the amphibole or as an exchangeable element in the chrysotile
43 (Douguet et al., 1997; Virta, 2002). The iron encountered in asbestos is able to catalyze many
44 reactions, such as lipid peroxidation, oxygen consumption, ROS formation, and DNA damage,
45 precursors for tumor development (Toyokuni, 2009; Valko et al., 2015). The various types of
46 toxic effects in humans include fibrogenesis and stomach, ovarian, and lung cancer (Scherpereel,
47 2016). Due to the associated health problems, asbestos was banned after more than 30 years of
48 intense use. As a consequence, we are confronted with the generation of a vast amount of
49 asbestos-containing waste (ACW).

50 Today, most waste is packed and deposited in controlled landfills, an inexpensive
51 technique that does not remove the toxicity or potential health risk of waste related to fiber
52 inhalation (Spasiano and Pirozzi, 2017). ACW treated by stabilization aims to reduce the fibers
53 released, without modifying the crystalline structure, and ends up in landfills. The second type of
54 treatment, called inertization, consisting of thermal treatment (vitrification or microwave), or
55 hydrothermal, chemical, or mechanochemical treatment, involves the total transformation of the
56 fibers, providing an inert and harmless material, considered to be a resource. However, such
57 thermal treatment is expensive due to energy consumption and may release emissions into the
58 atmosphere. Chemical treatment requires chemical products and their disposal, but has allowed
59 confirmation of the potential Mg released (Paolini et al., 2019). At the laboratory scale,
60 bioremediation experiments have been carried out on raw asbestos using various organisms

61 (fungi, lichens, or bacteria) or metabolites (organic acids or siderophores), whereas few studies
62 have focused on ACW. A few studies have focused on asbestos-cement waste, whereas none
63 have investigated biological treatment of flocking asbestos waste (FAW), of which the matrix
64 composition differs, containing gypsum to allow flocking to walls instead of cement. For
65 example, lichens are able to cover asbestos-cement roofs and modify the surface reactivity,
66 leading to bioavailable iron and magnesium liberation (Favero-Longo et al., 2009). Moreover,
67 modification of the fibers has been described, by which they became amorphous, due to oxalic
68 acid production (Favero-Longo et al., 2005) and nitrous and nitric acid produced by nitrifying
69 bacteria, such as *Nitrosomonas* and *Nitrobacter*, inducing corrosion of a cement-roof, thus
70 increasing porosity and reducing the strength of the material (Wasserbauer et al., 1988).
71 Moreover, Wang et al. (2010, 2011) also highlighted the porous structure of cement pipes after
72 microorganism colonization. They showed that acid-producing bacteria together with slime-
73 forming and heterotrophic bacteria contribute to corrosion of the inside surface of asbestos-
74 cement pipes of water distribution systems. Innovative bio-chemical processes have been
75 recently proposed, in which the cement phase is dissolved by lactic acid produced by
76 *Lactobacilli* or by dark fermentation, before hydrothermal end treatment (Balducci et al., 2012;
77 Spasiano et al., 2017). Siderophore production is a common mechanism shown in fungal and
78 bacterial weathering of native asbestos, leading to iron dissolution from fibers (Daghino et al.,
79 2010; Rajkumar et al., 2009). Siderophores are a well-known strategy that many living
80 organisms use to access iron, an essential element for growth. In mineral-bacterial weathering,
81 various siderophores have been implicated in iron dissolution, resulting in leaching of the
82 material, and pyoverdine (PVD) has been described to interact with iron-bearing silicates, such
83 as smectite (Ferret et al., 2014) and municipal waste-bottom ashe (Aouad et al., 2008). However,
84 this mechanism has not yet been investigated on ACW. We thus investigated the impact of the
85 bacterial siderophore PVD, produced by fluorescent *Pseudomonas*, on FAW. In building
86 construction, two types of FAW have been described, varying in texture, thickness, and density.
87 The first type used in this study contained 50 to 90% chrysotile fibers ($(\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4)$) and 10
88 to 50% gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), whereas the second contained 5 to 30% chrysotile fibers and 95
89 to 70% gypsum. Amphibole fibers, such as amosite ($(\text{Fe}, \text{Mg})_7\text{Si}_8\text{O}_{22}(\text{OH})_2$), have also
90 sometimes been combined with FAW. The ability of pyoverdine to extract iron from fiber has

91 already shown on chrysotile and amphibole raw asbestos. However, two main difficulties such as
92 the iron pollution present in the matrix and the fiber accessibility may interfere with the iron
93 extraction by pyoverdine.

94 Among the 100 PVDs so far described by siderotyping fluorescent *Pseudomonas*, their
95 affinity for iron is not yet known. We therefore tested several representatives from the PVD
96 laboratory collection to determine their ability to scavenge iron from ACW and selected the most
97 efficient. Iron removal was tested as a function of PVD concentration and the optimum time for
98 FAW alteration determined. Long-term alteration experiments by the best identified PVDs
99 determined the iron extraction limit.

100

101 **2. Experimental**

102 **2.1 Asbestos wastes preparation**

103 Two types of flocking asbestos wastes were used, chrysotile-gypsum and amosite-gypsum.
104 These wastes come from the asbestos removal site of Jussieu University of Paris and kindly
105 provided by the Mediterranean Company of Zeolites (SOMEZ). To obtain amosite-gypsum,
106 FAW samples were incubated in an acidic bath with sulfuric acid (8 mol/L), until total
107 dissolution of the chrysotile brucite layer. All asbestos samples (0.2 g) were sterilized by
108 autoclave (121°C for 20 min) and incubated at 70°C for 14 days before experiments. Before each
109 experiment, asbestos samples were washed with 20 mL of sterile succinate medium to remove
110 free Fe and Mg.

111

112 **2.2 Bacterial strains**

113 Bacteria used in this study were listed in table 1. Strains were stored in LB-20 % glycerol
114 stock at -80°C. *Pseudomonas* cells were grown routinely overnight at 30°C in Luria Bertani
115 medium (LB) under shaking condition (220 rpm).

116

117 **2.3 Production of pyoverdines**

118 1 mL of overnight LB culture of *Pseudomonas* strains was centrifuged (3 min at 9871 g).
119 For iron starved cells, the iron-deficient succinate medium (composition in g.L⁻¹: K₂HPO₄, 6.0;

120 KH_2PO_4 , 3.0; $(\text{NH}_4)_2\text{SO}_4$, 1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; sodium succinate, 4.0; pH adjusted to 7.0 by
121 addition of NaOH), sterilized by 0.2 μm filtration, was used (Meyer and Abdallah, 1978). The
122 pellet, washed twice with 1 ml of sterile succinate medium, was suspended in 1 ml of sterile
123 succinate medium and inoculated in 15 mL of sterile succinate medium, incubated at 30°C under
124 shaking condition (220 rpm). After 24 h of growth, 7.5 mL of this culture was transferred in an
125 Erlenmeyer flask containing 1 L of sterile succinate medium. After 24 h of incubation at 30°C
126 under shaking condition (220 rpm), the culture was centrifuged (2664 g for 40 min). The
127 supernatant was filtered twice with glass microfibre filters (Whatman, GF/C) and once on
128 nitrocellulose filter (0.45 μm porosity). To determine pyoverdine concentration, OD_{400} was
129 measured with a Specord 205 spectrophotometer (Analytik Jena). When needed, the PVD
130 concentration was adjusted with purified PVD according to the method described so far.
131 Siderophore containing supernatant was loaded, after acidification (pH 6.0), on XAD-4 column,
132 washed with two volume of purified water and eluted with one volume of 50% ethanol. The
133 eluate was concentrated under vacuum on a rotary evaporator and lyophilized.

134

135 **2.4 Asbestos dissolution by pyoverdines**

136 To evaluate the effect of various PVDs on the dissolution of iron from chrysotile-gypsum
137 and amosite-gypsum, 20 mL of PVD-containing supernatants adjusted to 100 μM were incubated
138 with materials in 50 mL Falcon tubes. After 24 h of incubation at 30°C under shaking condition
139 (220 rpm), the assays were centrifuged 30 min at 9871 g to recover supernatants. Assays were
140 done in 3 to 5 replicates.

141 Kinetics of iron dissolution from chrysotile-gypsum in 20 mL of PVD-containing
142 supernatant of *P. aeruginosa* and *P. mandelii* respectively adjusted to 100 μM were followed on
143 24 hours with regular sampling of the solution at 1, 2, 4, 6, 8 and 24 h. Assays were done in 5
144 replicates.

145 Long-term alteration of chrysotile-gypsum by PVD-containing supernatant of *P.*
146 *aeruginosa* and *P. mandelii* was performed by renewal cycles at 30°C under shaking condition
147 (220 rpm). Nine cycles of 24 hours and seven cycles of 96 h were realized in the presence of 100
148 μM of PVD-containing supernatant, followed by a 24 and a 96 h cycles with 200 μM of PVD-
149 containing supernatant. Assays were done in 5 replicates.

150 To determine the effect of pyoverdine concentrations produced by *P. mandelii* on iron and
151 magnesium dissolution from chrysotile-gypsum, material was incubated during 48 h at 30°C
152 under shaking condition (220 rpm) with 20 mL of supernatant adjusted to 25, 50, 100, 150 and
153 200 µM. Assays were done in 5 replicates.

154 Materials were eliminated from supernatants by filtration (0.22 µm porosity). Iron and
155 magnesium content were measured depending on the experiment.

156

157 **2.5 Determination of Fe and Mg contents**

158 In order to determine iron content, we used the bathophenanthroline method, which is a
159 sensitive colorimetric method for determining ferrous iron content. To determine the total iron,
160 ferric iron was converted to ferrous iron by thioglycolic acid. Each sample was analyzed in
161 triplicate. 20 µL of sample were dispensed into each well of a 96-well plate (Greiner, PS flat-
162 bottomed microplate). 40 µL of saturated sodium acetate (5.5 M) (Sigma-Aldrich), 80 µL of cold
163 distilled water, 10 µL of a thioglycolic acid solution diluted 10 times in water (Sigma-Aldrich)
164 and 10 µL of bathophenanthroline (5 mg.mL⁻¹) (Sigma-Aldrich) were additionned per well.
165 After shaking, microplates were stored overnight at 4°C. Absorbance was measured at 535 nm in
166 a Tecan microplate reader (Infinite M200, Tecan) and iron concentration (mg.L⁻¹) was
167 determined by reference to a standard curve.

168 For the magnesium content, calmagite method was used. Magnesium formed a coloured
169 complex with calmagite. EGTA (Ethylene Glycol Tetraacetic Acid) was used to eliminate any
170 calcium interference. Each sample was analyzed in triplicate. To prepare the reactive solution,
171 one volume of reagent 1 (1 mol.L⁻¹ of 2-Methyl-2-Amino-1-Propanol and 215 µmol.L⁻¹ of
172 EGTA) with one volume of reagent 2 (300 µmol.L⁻¹ of calmagite) were mixed. 3 µL of sample
173 were dispensed into each well of a 96-well plate (Greiner, PS flat-bottomed microplate) and 300
174 µL of reactive solution mixed were added. After shaking and 30 seconds of incubation,
175 absorbance was measured at 500 nm in a Tecan microplate reader (Infinite M200, Tecan) and
176 magnesium concentration (mg.L⁻¹) was determined by reference to a standard curve.

177

178 **2.6 STEM-EDX of asbestos fibers**

179 TEM (Transmission Electron Microscopy) images were recorded with a JEOL 2100
180 microscope, with a 200 kV potential applied on a LaB6 filament as an electron source.
181 Resolution of the TEM is 0.21 nm. TEM mapping was performed in STEM (Scanning
182 transmission electron microscopy) mode (resolution 2 nm) and using an SSD-EDX (Silicon Drift
183 Detector-Energy Dispersive X-ray) spectrometer to determine the chemical composition.

184

185 **2.7 Statistical analysis**

186 Results presented in all figures correspond to mean values of three to five replicates.
187 Significant statistical differences among values were determined using Kruskal-Wallis test
188 (RStudio v1.0.153) followed by a Conover post-hoc analysis. Values with the same letter are not
189 significantly different at $p = 0.05$.

190

191 **3. Results and discussion**

192 **3.1 Efficiency of pyoverdines on iron removal from flocking asbestos wastes**

193 PVDs, yellow-green fluorescent pigments, are composed of three moieties: (i) a peptide
194 chain varying in the nature, number, and order of amino acids, (ii) a dihydroxyquinoline
195 chromophore, conferring the yellow-green color of the molecule, and (iii) an acyl chain radical
196 made of dicarboxylic acid residue which can be either succinate, malate, or their amide forms, or
197 alpha-ketoglutarate or glutamate, depending on the producing strain and growth conditions.
198 Based on these structural differences, PVDs have been purified from various *Pseudomonas*
199 strains to obtain a large family composed of more than 100 different structures, depending on
200 their peptide chain and radical R (Meyer et al., 1998). PVD binds to Fe^{3+} in a 1:1 stoichiometry
201 with a stability constant for Fe(III) of approximately 10^{32} M^{-1} for PVD produced by *P.*
202 *aeruginosa* PAO1 (Albrecht-Gary et al., 1994). From this bank, we selected nine PVDs produced
203 by various *Pseudomonas* species based on their different isoelectrofocusing profile related to the
204 length, nature, and presence of cycle rings in the peptide chain (Table 1). These selected PVDs
205 were tested to investigate potential differences in their ability to chelate iron. We previously
206 showed that purified PVDs can extract iron from the three native asbestos types, chrysotile,
207 amosite, and crocidolite, and with the same efficiency as PVD-containing supernatant (David et

208 al., 2019). Therefore, we used PVD-containing supernatants to determine the effect of these
209 siderophores on FAW, chrysotile-gypsum, and amosite-gypsum (Fig. 1). The nine selected PVDs
210 (at a concentration of 100 μM) were incubated with each FAW for 24 h. The control medium
211 (sterile succinate medium) had no visible effect on iron dissolution from waste, whereas all
212 tested PVDs exhibited significant iron extraction from both types of waste, ranging from 0.24
213 mg.L^{-1} to 1.22 mg.L^{-1} iron dissolved for chrysotile-gypsum and 0.63 to 5.39 mg.L^{-1} for amosite-
214 gypsum. For chrysotile-gypsum, 25% of the siderophores were in the PVD-Fe complex form,
215 whereas PVD was almost saturated at 100% for amosite-gypsum, a factor that may have limited
216 material weathering. A higher amount of extracted iron was obtained for amosite-gypsum, which
217 is consistent with the chemical composition of this material, which contains more iron than
218 chrysotile-gypsum. Iron removal was not the same for all PVDs. Moreover, the PVD which
219 extracted the most iron was different, depending on the type of waste: PVD from *P. mandelii* for
220 chrysotile-gypsum, with 1.22 mg.L^{-1} , and *P. fluorescens* for amosite-gypsum, with 5.39 mg.L^{-1} .
221 In addition, the amount of iron extracted from samples incubated in fresh supernatants for 48 h
222 was the same as that measured after 24 h (data not shown). Studies examining siderophore-
223 mediated iron dissolution from chrysotile waste are lacking. A few studies have focused on the
224 effect of the commercial bacterial siderophore desferrioxamine, produced by *Streptomyces*
225 *pilosus*, on asbestos fibers. Iron removal from crocidolite and amosite by this siderophore is also
226 effective (Chao and Aust, 1994). Moreover, Mohanty et al. (2018) showed that desferrioxamine
227 releases exchangeable and structural iron from chrysotile fibers in a concentration-dependent
228 manner. Other studies have shown that fungal strains can extract iron from asbestos, likely
229 promoted by siderophores (Daghino et al., 2008). Mohanty et al. (2018) showed that
230 desferrioxamine was as effective as the fungi siderophore, ferrichrome, in removing iron from
231 chrysotile fibers.

232

233 **3.2 Kinetics of iron extraction from chrysotile-gypsum by pyoverdines**

234 We then focused on the most representative ACW among waste, chrysotile-gypsum. Based
235 on the results show in Fig. 1, we carried out waste alteration using the two PVD-containing
236 supernatants from *P. aeruginosa* (PVD_{aeruginosa}) and *P. mandelii* (PVD_{mandelii}), adjusted to a
237 final concentration of 100 μM (Fig. 2). We sampled and measured iron solubilization during the

238 first 8 h and at the end of the incubation to better understand the iron extraction kinetics. The
239 kinetic profiles of dissolution by the two PVDs were very similar and achieved the same iron
240 extraction (0.9 mg.L^{-1}) at the end of the experiment, consistent with the results presented in Fig.
241 1. We detected dissolved iron for PVD_{mandelii} and PVD_{aeruginosa} after only 1 h of contact (0.12
242 and 0.17 mg.L^{-1} , respectively), which progressively increased during the next 8 h of dissolution
243 (0.59 and 0.54 mg.L^{-1} , respectively). The same time scale of 24 h was used by Mohanty et al.
244 (2018) to reach an iron dissolution equilibrium for chrysotile fiber weathering by
245 desferrioxamine. However, we cannot compare the iron concentrations because our experiment
246 focused on chrysotile waste embedded in gypsum for flocking purposes in housing, whereas that
247 of Mohanty *et al.* used raw asbestos fibers.

248

249 **3.3 Long-term alteration of chrysotile-gypsum by pyoverdines**

250 Our experiments clearly show that the optimum time for chrysotile-gypsum alteration is 24
251 h without renewal of the medium. We conducted a second set of experiments, with periodic
252 medium renewal, for 42 days on FAW to determine the limit of iron extraction by PVDs
253 produced by *P. aeruginosa* and *P. mandelii*. The supernatant was replaced by fresh apo-PVD
254 supernatant at each cycle. PVD_{aeruginosa} and PVD_{mandelii} showed equivalent iron release over
255 time, which is consistent with the previous results. Succinate medium alone had no effect on iron
256 dissolution, as only low iron concentrations were detected in the control (0.01 to 0.08 mg.L^{-1})
257 (Fig. 3). During the 24 h cycles (T24-1 to T24-8), using siderophore concentrations of $100 \mu\text{M}$,
258 iron extraction decreased from 0.92 to 0.28 mg.L^{-1} for PVD_{aeruginosa} and 0.88 to 0.22 mg.L^{-1}
259 for PVD_{mandelii}. We observed an unexpected increase to 0.42 for PVD_{aeruginosa} and 0.37 for
260 PVD_{mandelii} for T24-9, due to a longer stand-by period (a week) between T24-8 and T24-9 than
261 for the other cycles, for which the duration was < 72 h. Thus, the cycles were extended to 96 h
262 for seven cycles. At T96-1, iron dissolution was 0.55 mg.L^{-1} for PVD_{aeruginosa} and 0.45 mg.L^{-1}
263 for PVD_{mandelii}. After T96-2, released iron decreased to 0.24 mg.L^{-1} for PVD_{aeruginosa} and 0.25
264 mg.L^{-1} for PVD_{mandelii} and remained constant between T96-5 and T96-7. Then, we incubated

265 the chrysotile-gypsum in a 200 μM PVD-containing supernatant for 24 and 96 h to determine
266 whether additional iron could be extracted. However, the concentration of solubilized iron was
267 very low (0.17 for PVD_{aeruginosa} and 0.09 $\text{mg}\cdot\text{L}^{-1}$ for PVD_{mandelii} after 96 h). The waste
268 contains chrysotile, with a theoretical composition of $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$, which is a sheet silicate
269 composed of inner tetrahedral silicate layers and outer magnesium oxide octahedral layers
270 (brucite). Since asbestos originated from soils where iron is highly abundant, magnesium in the
271 outer layer may be replaced by ferrous and ferric iron. The same phenomenon may occur in the
272 inner silicate layer, where silicon may be exchanged by ferric iron only (Pollastri et al., 2015). In
273 our study, some iron appeared to be more easily solubilized during short-term assay (24 h),
274 whereas we needed to increase the exposure time to 96 h to continue iron dissolution. It is well
275 known that siderophore-mediated iron removal leads to siderophore adsorption on the iron-
276 bearing mineral surface. For example, PVD has already been shown to dissolve structural iron
277 from smectite upon contact (Ferret et al., 2014). A few studies have focused on siderophore
278 dissolution mechanism for mineral asbestos. Mohanty et al. (2018) studied iron release from
279 chrysotile fibers, depending on the concentration of desferrioxamine in long-term experiments
280 with no solution renewal. The low concentration of desferrioxamine (1 μM) released some iron
281 from the surface of the brucite layer, whereas increasing the concentration of desferrioxamine to
282 10 μM led to better surface coverage. Exhaustion of surface sites could provide easier access to
283 the iron present in the silica tetrahedron. In our experiment, the concentration of both
284 siderophores remained predominantly constant (100 μM) and the solutions were renewed at
285 regular intervals of 24 h or 96 h. Our results tend to point to the same conclusion as those of
286 Mohanty et al. (2018), in which short renewal cycles appear to release iron from the brucite
287 layer, leaching the fiber surface and potentially providing access to iron from the silica layer.
288 The increase in iron removal by the 96 h renewal cycles could be explained by two mechanisms:
289 i) better siderophore surface coverage and the ii) release of iron from the silica layer. As iron
290 dissolution from the silica tetrahedron could be slow, our long renewal cycles may have
291 facilitated such weathering.

292 Analysis of chrysotile fibers by STEM-EDX (Fig. 4) showed a large decrease of iron
293 content after long-term alteration with both PVDs and confirms that iron was removed from the

294 structure. Interestingly, the silica content tended also to decrease after PVD contact. These
295 results confirm active dissolution driven by the siderophores.

296

297 **3.4 Effect of pyoverdine concentrations on iron and magnesium dissolution from** 298 **chrysotile-gypsum**

299 Various concentrations of PVD_{mandelii} were added to FAW over 48 h to evaluate iron and
300 magnesium release. We observed iron removal from chrysotile fibers in a dose-dependent
301 manner (Fig. 5A). The control (succinate medium alone) showed only weak dissolution (0.034
302 mg.L⁻¹), whereas iron release was significantly higher in the presence of PVD from 0.30 mg.L⁻¹
303 at 25 μM to 0.96 mg.L⁻¹ at 150 μM. The amount of iron released by 200 μM PVD was similar to
304 that released by 150 μM, showing that the PVD concentration was not a limiting factor. Indeed,
305 10% of the siderophore was in a PVD-Fe complex form at 200 μM. Thus, the reaction involved a
306 proportion of apo-PVD which could be high enough to extract iron, indicating that the limiting
307 factor could be the availability of iron. Mohanty et al. (2018) showed that iron removal from
308 chrysotile fibers increased if the concentration of desferrioxamine was raised from 1 to 10 μM.
309 Our results confirm this study and suggest that iron release is limited by iron availability.
310 Siderophores are well known for their high affinity for iron and recently Schalk et al. (2011)
311 demonstrated that other metals can also be chelated by the PVD of *P. aeruginosa*. However, no
312 data are available on the capacity of PVD to complex magnesium. Given that the studied FAW
313 contains chrysotile with an external brucite layer composed of magnesium, fiber alteration may
314 result in magnesium solubilization. We thus evaluated the effect of PVD_{mandelii} on magnesium
315 dissolution as well as that of iron (Fig. 5B). We observed no effect on magnesium dissolution at
316 any tested concentrations. A relatively high magnesium concentration was measured in the
317 control (5.5 mg.L⁻¹), with no significative difference from the amount of magnesium dissolved
318 by PVD_{mandelii} (3.85 mg.L⁻¹ to 6.89 mg.L⁻¹), regardless of the concentration tested. pH is a
319 well-known parameter that has been described to influence the dissolution of the chrysotile
320 brucite layer (Choi and Smith, 1972; Gronow, 1987). An acidic environment could strongly
321 favor magnesium ion leaching, whereas slower dissolution kinetics may occur at neutral pH. All

322 solutions in our study were adjusted to pH 7 and thus measured magnesium release may have
323 been due to the pH.

324

325

326 **4. Conclusions**

327 We investigated the efficiency of various PVDs to scavenge iron from FAW and the ability
328 of the best identified PVDs to degrade asbestos waste. Our results showed a significant iron
329 extraction from FAW by all tested PVDs but with differences in efficiency according to the
330 pyoverdines and the type of waste. PVD from *P. mandelii* extracted the higher amount of iron
331 from chrysotile-gypsum, while PVD from *P. fluorescens* was the most efficient for amosite-
332 gypsum. The kinetic profiles of iron extraction from chrysotile-gypsum by these pyoverdines
333 highlighted a time-dependent dissolution. A long-term alteration of chrysotile-gypsum by
334 PVD_{aeruginosa} and PVD_{mandelii} revealed an extraction limit after renewal cycles for a total of 42
335 days leading to a large decrease of iron content from chrysotile fibers structure. Moreover,
336 PVD_{mandelii} extracted iron from chrysotile-gypsum in concentration-dependent manner. Our
337 research identified efficient pyoverdines in biodegradation of asbestos waste. The resulting PVD-
338 Fe complex could be exploited as a valuable iron source. Further investigations might allow to
339 consider the development of an eco-friendly asbestos treatment, using pyoverdines, in order to
340 reduce asbestos-related environmental and health problems.

341

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346

347 **References**

348 Albrecht-Gary, A.-M., Blanc, S., Rochel, N., Ocaktan, A.Z., Abdallah, M.A., 1994. Bacterial
349 iron transport: Coordination properties of pyoverdin PaA, a peptidic siderophore of
350 *Pseudomonas aeruginosa*. Inorg. Chem. 33, 6391–6402. <https://doi.org/10.1021/ic00104a059>

- 351 Aouad, G., Crovisier, J.-L., Damidot, D., Stille, P., Hutchens, E., Mutterer, J., Meyer, J.-M.,
 352 Geoffroy, V.A., 2008. Interactions between municipal solid waste incinerator bottom ash and
 353 bacteria (*Pseudomonas aeruginosa*). *Sci. Total Environ.* 393, 385–393.
 354 <https://doi.org/10.1016/j.scitotenv.2008.01.017>
- 355 Balducci, G., Foresti, E., Lelli, M., Lesci, I.G., Marchetti, M., Pierini, F., Roveri, N., 2012.
 356 Process for treating an asbestos containing material. EP2428254A1.
- 357 Briskot, G., Taraz, K., Budzikiewicz, H., 1989. Bacterial constituents, XXXVII. pyoverdinin-type
 358 siderophores from *Pseudomonas aeruginosa*. *Liebigs Annalen der Chemie* 1989, 375–384.
 359 <https://doi.org/10.1002/jlac.198919890164>
- 360 Budzikiewicz, H., Schröder, H., Taraz, K., 2014. Zur biogenese der *Pseudomonas*-siderophore:
 361 Der nachweis analoger strukturen eines pyoverdinin-desferriferribactin-paares. *Zeitschrift für*
 362 *Naturforschung C* 47, 26–32. <https://doi.org/10.1515/znc-1992-1-206>
- 363 Chao, C.C., Aust, A.E., 1994. Effect of long-term removal of iron from asbestos by
 364 desferrioxamine B on subsequent mobilization by other chelators and induction of DNA single-
 365 strand breaks. *Arch. Biochem. Biophys.* 308, 64–69. <https://doi.org/10.1006/abbi.1994.1009>
- 366 Choi, I., Smith, R.W., 1972. Kinetic study of dissolution of asbestos fibers in water. *J. Colloid*
 367 *Interface Sci.* 40, 253–262. [https://doi.org/10.1016/0021-9797\(72\)90014-8](https://doi.org/10.1016/0021-9797(72)90014-8)
- 368 Dabboussi, F., Hamze, M., Singer, E., Geoffroy, V., Meyer, J.-M., Izard, D., 2002. *Pseudomonas*
 369 *mosselii* sp. nov., a novel species isolated from clinical specimens. *Int. J. Syst. Evol. Microbiol.*
 370 52, 363–376. <https://doi.org/10.1099/00207713-52-2-363>
- 371 Daghino, S., Martino, E., Perotto, S., 2010. Fungal weathering and implications in the
 372 solubilization of metals from soil and from asbestos fiber. *Current research, technology and*
 373 *education topics in applied microbiology and microbial biotechnology* 1, 329–338.
- 374 Daghino, S., Martino, E., Vurro, E., Tomatis, M., Girlanda, M., Fubini, B., Perotto, S., 2008.
 375 Bioweathering of chrysotile by fungi isolated in ophiolitic sites. *FEMS Microbiol Lett* 285, 242–
 376 249. <https://doi.org/10.1111/j.1574-6968.2008.01239.x>
- 377 David, S.R., Ihiawakrim, D., Regis, R., Geoffroy, V.A., 2019. Iron removal from raw asbestos
 378 by siderophores-producing *Pseudomonas*. *J. Hazard. Mater.* 121563.
 379 <https://doi.org/10.1016/j.jhazmat.2019.121563>
- 380 Douguet, D., Carteron, H., Janiaud, P., Pinhas, N., 1997. Effets sur la santé des principaux types
 381 d'exposition à l'amiante. Institut national de la santé et de la recherche médicale (INSERM).
- 382 Favero-Longo, S.E., Castelli, D., Fubini, B., Piervittori, R., 2009. Lichens on asbestos–cement
 383 roofs: Bioweathering and biocovering effects. *J. Hazard. Mater.* 162, 1300–1308.
 384 <https://doi.org/10.1016/j.jhazmat.2008.06.060>
- 385 Favero-Longo, S.E., Turci, F., Tomatis, M., Castelli, D., Bonfante, P., Hochella, M.F.,
 386 Piervittori, R., Fubini, B., 2005. Chrysotile asbestos is progressively converted into a non-fibrous
 387 amorphous material by the chelating action of lichen metabolites. *J. Environ. Monit.* 7, 764–766.
 388 <https://doi.org/10.1039/B507569F>

- 389 Ferret, C., Sterckeman, T., Cornu, J.-Y., Gangloff, S., Schalk, I.J., Geoffroy, V.A., 2014.
390 Siderophore- promoted dissolution of smectite by fluorescent *Pseudomonas*. Environ.
391 Microbiol. Rep. 6, 459–467. <https://doi.org/10.1111/1758-2229.12146>
- 392 Gronow, J.R., 1987. The dissolution of asbestos fibres in water. Clay Minerals 22, 21–35.
393 <https://doi.org/10.1180/claymin.1987.022.1.03>
- 394 Hohlneicher, U., Hartmann, R., Taraz, K., Budzikiewicz, H., 2014. Pyoverdine, ferribactin,
395 azotobactin - a new triade of siderophores from *Pseudomonas chlororaphis* ATCC 9446 and its
396 relation to *Pseudomonas fluorescens* ATCC 13525. Zeitschrift für Naturforschung C 50, 337–
397 344. <https://doi.org/10.1515/znc-1995-5-602>
- 398 Jülich, M., Taraz, K., Budzikiewicz, H., Geoffroy, V., Meyer, J.-M., Gardan, L., 2014. The
399 structure of the pyoverdine isolated from various *Pseudomonas syringae* pathovars. Zeitschrift für
400 Naturforschung C 56, 687–694. <https://doi.org/10.1515/znc-2001-9-1003>
- 401 Meyer, J.M., Abdallah, M.A., 1978. The fluorescent pigment of *Pseudomonas fluorescens*:
402 Biosynthesis, purification and physicochemical properties. Microbiology 107, 319–328.
403 <https://doi.org/10.1099/00221287-107-2-319>
- 404 Meyer, J.-M., Gruffaz, C., Raharinosy, V., Bezverbnaya, I., Schäfer, M., Budzikiewicz, H., 2008.
405 Siderotyping of fluorescent *Pseudomonas*: Molecular mass determination by mass spectrometry
406 as a powerful pyoverdine siderotyping method. Biometals 21, 259–271.
407 <https://doi.org/10.1007/s10534-007-9115-6>
- 408 Meyer, J.-M., Stintzi, A., Coulanges, V., Shivaji, S., Voss, J.A., Taraz, K., Budzikiewicz, H.,
409 1998. Siderotyping of fluorescent pseudomonads: Characterization of pyoverdines of
410 *Pseudomonas fluorescens* and *Pseudomonas putida* strains from Antarctica. Microbiology 144,
411 3119–3126. <https://doi.org/10.1099/00221287-144-11-3119>
- 412 Mohanty, S.K., Gonneau, C., Salamatipour, A., Pietrofesa, R.A., Casper, B., Christofidou-
413 Solomidou, M., Willenbring, J.K., 2018. Siderophore-mediated iron removal from chrysotile:
414 Implications for asbestos toxicity reduction and bioremediation. J. Hazard. Mater. 341, 290–296.
415 <https://doi.org/10.1016/j.jhazmat.2017.07.033>
- 416 Paolini, V., Tomassetti, L., Segreto, M., Borin, D., Liotta, F., Torre, M., Petracchini, F., 2019.
417 Asbestos treatment technologies. J. Mater. Cycles Waste Manag. 21, 205–226.
418 <https://doi.org/10.1007/s10163-018-0793-7>
- 419 Persmark, M., Frejd, T., Mattiasson, B., 1990. Purification, characterization, and structure of
420 pseudobactin 589 A, a siderophore from a plant growth promoting *Pseudomonas*. Biochemistry
421 29, 7348–7356. <https://doi.org/10.1021/bi00483a026>
- 422 Pollastri, S., D’Acapito, F., Trapananti, A., Colantoni, I., Andreozzi, G.B., Gualtieri, A.F., 2015.
423 The chemical environment of iron in mineral fibres. A combined X-ray absorption and
424 Mössbauer spectroscopic study. J. Hazard. Mater. 298, 282–293.
425 <https://doi.org/10.1016/j.jhazmat.2015.05.010>
- 426 Rajkumar, M., Vara Prasad, M.N., Freitas, H., Ae, N., 2009. Biotechnological applications of

427 serpentine soil bacteria for phytoremediation of trace metals. Crit. rev. biotechnol. 29, 120–130.
428 <https://doi.org/10.1080/07388550902913772>

429 Schalk, I.J., Hannauer, M., Braud, A., 2011. New roles for bacterial siderophores in metal
430 transport and tolerance. Environ. Microbiol. 13, 2844–2854. <https://doi.org/10.1111/j.1462->
431 2920.2011.02556.x

432 Scherpereel, A., 2016. Amiante et pathologie respiratoire. La Presse Médicale, Médecine et
433 environnement 45, 117–132. <https://doi.org/10.1016/j.lpm.2015.12.011>

434 Spasiano, D., Luongo, V., Petrella, A., Alfè, M., Pirozzi, F., Fratino, U., Piccinni, A.F., 2017.
435 Preliminary study on the adoption of dark fermentation as pretreatment for a sustainable
436 hydrothermal denaturation of cement-asbestos composites. J. Clean. Prod. 166, 172–180.
437 <https://doi.org/10.1016/j.jclepro.2017.08.029>

438 Spasiano, D., Pirozzi, F., 2017. Treatments of asbestos containing wastes. J. Environ. Manage.
439 204, Part 1, 82–91. <https://doi.org/10.1016/j.jenvman.2017.08.038>

440 Teintze, M., Hossain, M.B., Barnes, C.L., Leong, J., Van der Helm, D., 1981. Structure of ferric
441 pseudobactin: A siderophore from a plant growth promoting *Pseudomonas*. Biochemistry 20,
442 6446–6457. <https://doi.org/10.1021/bi00525a025>

443 Toyokuni, S., 2009. Mechanisms of asbestos-induced carcinogenesis. Nagoya J. Med. Sci. 71, 1–
444 10.

445 Valko, M., Jomova, K., Rhodes, C.J., Kuča, K., Musílek, K., 2015. Redox- and non-redox-metal-
446 induced formation of free radicals and their role in human disease. Arch. Toxicol. 90, 1–37.
447 <https://doi.org/10.1007/s00204-015-1579-5>

448 Virta, R.L., 2002. Asbestos: Geology, mineralogy, mining, and uses. US Department of the
449 Interior. US Geological Survey.

450 Wang, D., Cullimore, D.R., 2010. Bacteriological challenges to asbestos cement water
451 distribution pipelines. J. Environ. Sci. 22, 1203–1208. <https://doi.org/10.1016/S1001->
452 0742(09)60239-4

453 Wang, D., Cullimore, R., Hu, Y., Chowdhury, R., 2011. Biodeterioration of asbestos cement
454 (AC) pipe in drinking water distribution systems. Int. Biodeterior. Biodegrad. 65, 810–817.
455 <https://doi.org/10.1016/j.ibiod.2011.05.004>

456 Wasserbauer, R., Zádák, Z., Novotný, J., 1988. Nitrifying bacteria on the asbestos-cement roofs
457 of stable buildings. International Biodeterioration 24, 153–165. <https://doi.org/10.1016/0265->
458 3036(88)90045-0

459 Wong-Lun-Sang, S., Bernardini, J.-J., Hennard, C., Kyslík, P., Dell, A., Abdallah, M.A., 1996.
460 Bacterial siderophores: Structure elucidation, 2D 1H and 13C NMR assignments of pyoverdins
461 produced by *Pseudomonas fluorescens* CHAO. Tetrahedron Lett. 37, 3329–3332.
462 [https://doi.org/10.1016/0040-4039\(96\)00569-2](https://doi.org/10.1016/0040-4039(96)00569-2)

463

465 FIGURE LEGENDS:

466

467 **Fig. 1.** Concentration of iron dissolved from flocking asbestos waste, chrysotile-gypsum (A) and
468 amosite-gypsum (B), in the presence of various pyoverdine-containing supernatants (SN) after
469 24 h of contact. Error bars indicate the standard errors of the means of three or five replicates.
470 Bars with the same letter are not significantly different ($p > 0.05$, Kruskal-Wallis test, three or
471 five replicates).

472

473 **Fig. 2.** Evolution of iron concentration extracted from chrysotile-gypsum waste over 24 h in the
474 presence of *Pseudomonas aeruginosa* or *Pseudomonas mandelii* pyoverdine-containing
475 supernatants (SN). Error bars indicate the standard errors of the means of five replicates.

476

477 **Fig. 3.** Iron removal from chrysotile-gypsum waste after renewal cycles of 24 or 96 h in the
478 presence of 100 μ M (T24-1 to T24-9 and T96-1 to T96-7) or 200 μ M (T24-C and T96-C)
479 *Pseudomonas aeruginosa* or *Pseudomonas mandelii* pyoverdine-containing supernatants (SN).
480 Error bars indicate the standard errors of the means of five replicates.

481

482 **Fig. 4.** STEM images and STEM mapping of chrysotile-gypsum fibers after renewal cycles of 24
483 or 96 h in the presence of succinate medium (a and b) or *Pseudomonas mandelii* pyoverdine-
484 containing supernatants (SN) (c and d). Large images obtained from the combination of the three
485 distributions of Mg, Si, and Fe with analysis areas (a and c). Atomic ratios of Mg/Si and Fe/Si (b
486 and d). (e) Comparison histogram after renewal cycles with succinate medium or *Pseudomonas*
487 *mandelii* pyoverdine-containing supernatants.

488

489 **Fig. 5.** Effect of various concentrations of pyoverdine produced by *Pseudomonas mandelii* on
490 iron (A) and magnesium (B) dissolution from chrysotile-gypsum waste after 48 h of contact.
491 Error bars indicate the standard errors of the means of five replicates. Bars with the same letter
492 are not significantly different ($p > 0.05$, Kruskal-Wallis test of five replicates).

493

494

Highlights

- Weathering efficiency varies according to the pyoverdines and the type of waste.
- Pyoverdines solubilize iron in a time-dependent manner.
- Chrysotile-gypsum weathering by pyoverdines decreases iron concentration in fibers.
- Iron removal from chrysotile-gypsum is concentration-dependent of pyoverdines.
- Pyoverdines could be used in bioremediation process of asbestos waste.

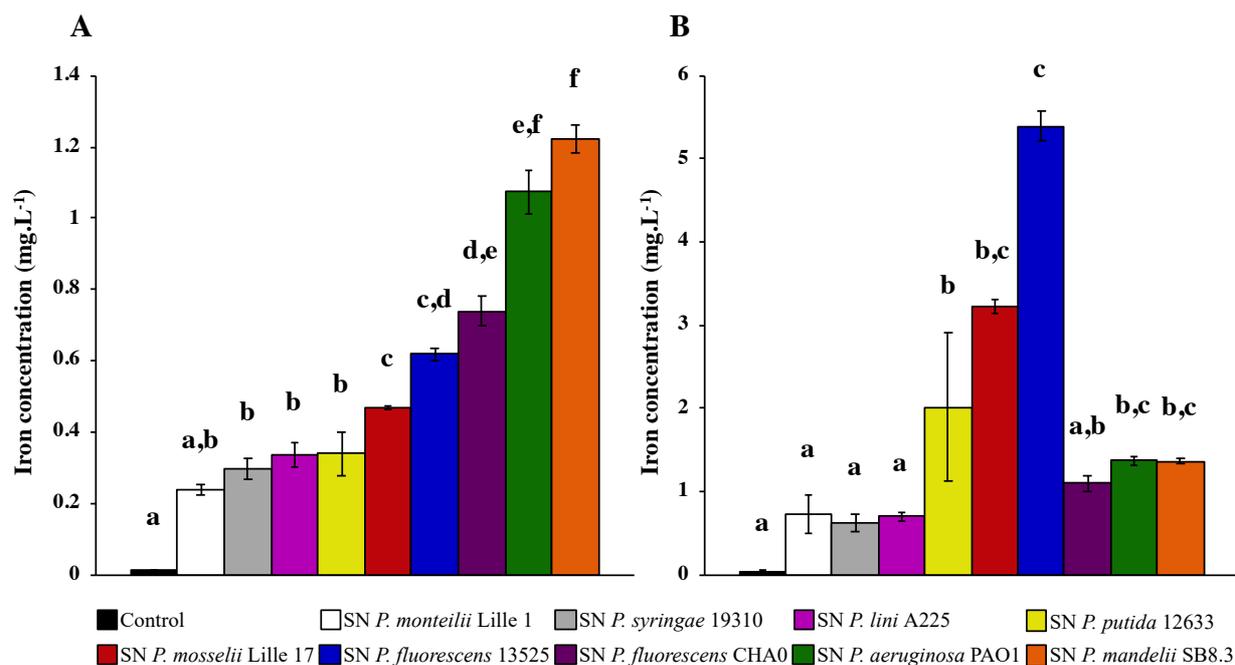
1 **Table 1.** List of pyoverdines used, with their molecular mass, isoelectrofocusing
 2 characteristics, and peptide composition.

Siderovars	Species	MM	Number of isoforms and pHi values	Pyoverdine peptide chain	References
Lille 1	<i>P. monteilii</i>	1 291	2 7.3/4.6	Asp-Lys-AcOHOrn-Ala-Ser-Ser-Gly-Ser-cOHOrn	Meyer et al. (2008)
Syr	<i>P. syringae</i>	1 123	2 5.0/4.0	ϵ Lys-OH <u>Asp</u> -Thr-(Thr-Ser-OH <u>Asp</u> -Ser)	Jülich et al. (2014)
B10/PL9/ 7SR1/A225	<i>P. lini</i>	989	3 7.5/5.2/4.6	ϵ Lys-OH <u>Asp</u> -Ala-a <u>Thr</u> -Ala-cOHOrn	Teintze et al. (1981)
12633	<i>P. putida</i>	1 336	3 4.6/4.2/4.0	Asp- ϵ Lys-OH <u>Asp</u> -Ser-Thr- <u>Ala</u> - <u>Glu</u> -Ser cOHOrn	Persmark et al. (1990)
Lille 17	<i>P. mosselii</i>	-	3 9.0/7.4/5.2	ND	Dabboussi et al. (2002)
13525	<i>P. fluorescens</i>	1 160	3 8.7/7.3/7.1	<u>Ser</u> -Lys-Gly-FOHOrn-(Lys-FOHOrn-Ser)	Hohlneicher et al. (2014)
CHA0	<i>P. fluorescens</i>	1 287	3 8.5/7.5/5.1	Asp-FOHOrn-Lys-(Thr- <u>Ala</u> - <u>Ala</u> -FOHOrn-Lys)	Wong-Lun-Sang et al. (1996)
PAO1	<i>P. aeruginosa</i>	1 333	2 8.8/7.0	<u>Ser</u> -Arg- <u>Ser</u> -FOHOrn-(Lys-FOHOrn-Thr-Thr)	Briskot et al. (1989)
SB8.3	<i>P. mandelii</i>	1 046	3 9.0/8.8/7.6	<u>Ala</u> -Lys-Thr- <u>Ser</u> -AOHOrn-cOHOrn	Budzikiewicz et al. (2014)

3 ϵ Lys: lys linked by its ϵ -NH₂; OHAsp: threo- β -hydroxy-aspartic acid; aThr: allo-Thr;
 4 cOHOrn: cyclo-hydroxy-ornithine; FOHOrn: δ N -formyl- δ N -hydroxy-ornithine; AOHOrn
 5 δ N -acetyl- δ N -hydroxy-ornithine.

6 Parentheses indicate cyclic structures. D-amino acids are underlined. A broken line means
 7 that the two enantiomers were detected among the underlined residues. ND: not determined.

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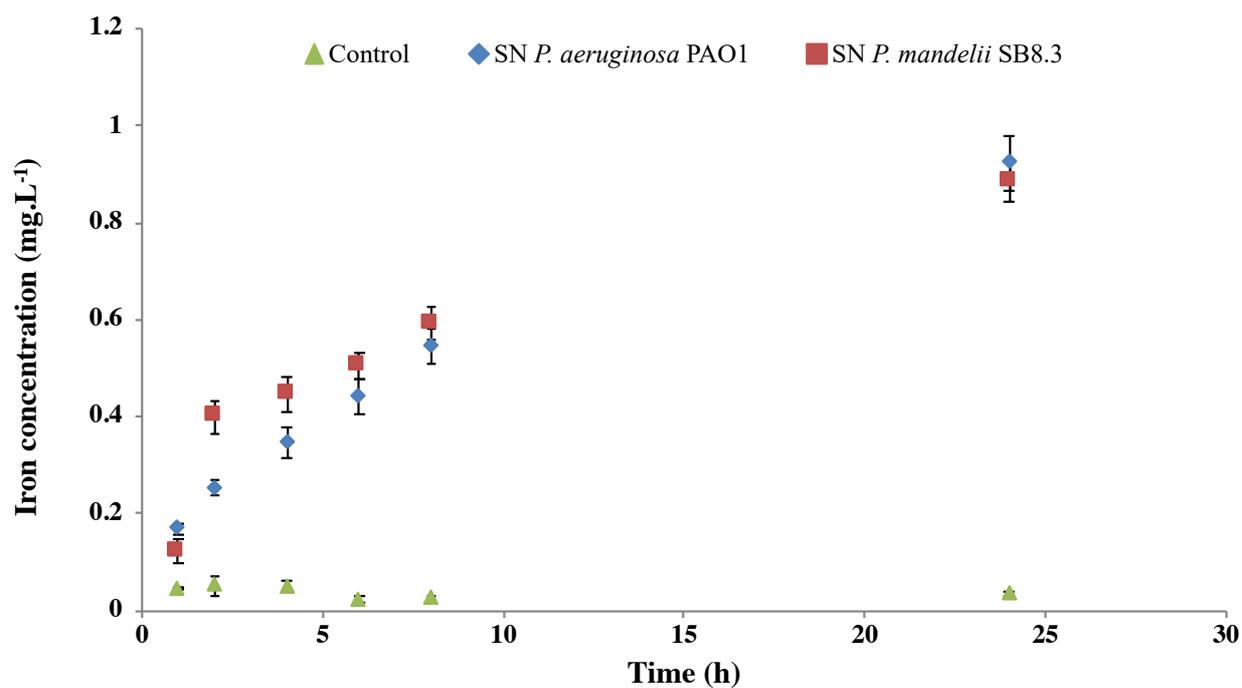
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3 **Fig. 1.** Concentration of iron dissolved from flocking asbestos waste, chrysotile-gypsum (A)
 4 and amosite-gypsum (B), in the presence of various pyoverdine-containing supernatants (SN)
 5 after 24 h of contact. Error bars indicate the standard errors of the means of three or five
 6 replicates. Bars with the same letter are not significantly different ($p > 0.05$, Kruskal-Wallis
 7 test, three or five replicates).

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9 **In color online**

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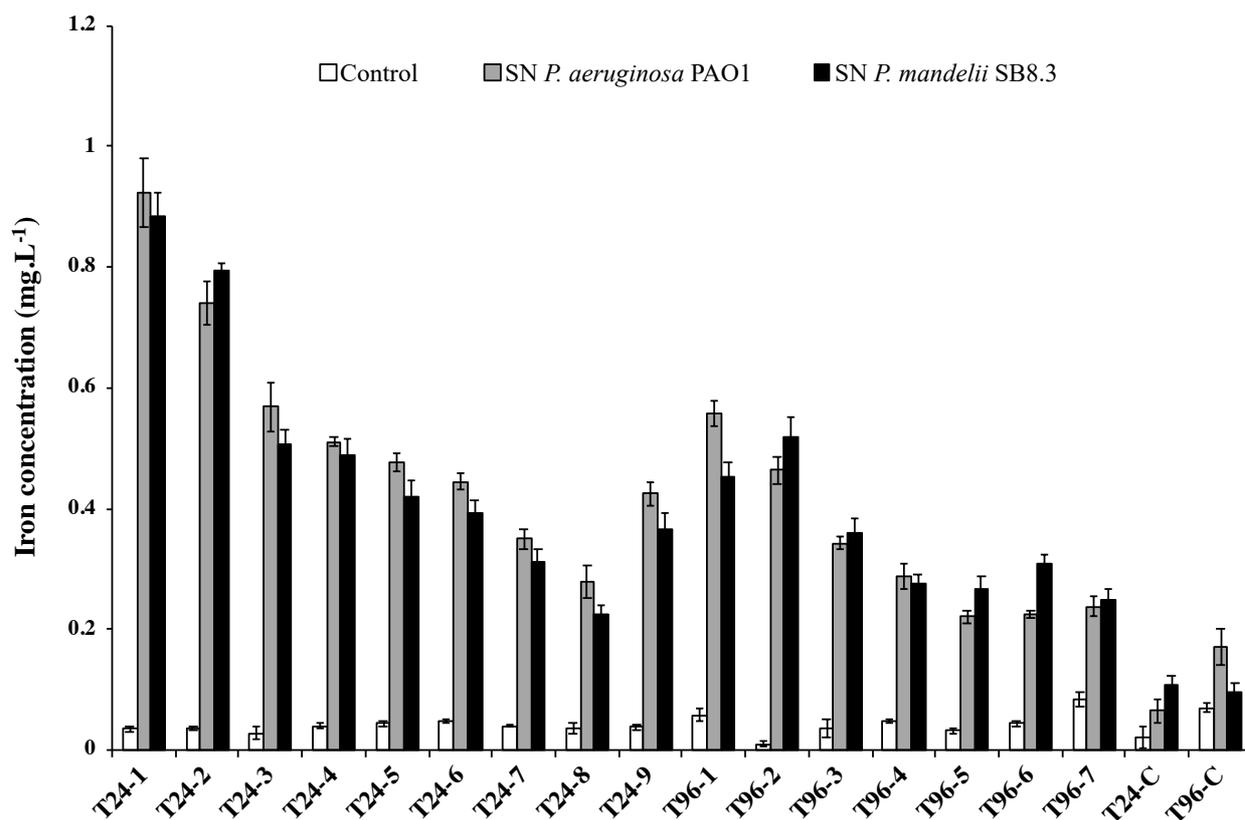
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3 **Fig. 2.** Evolution of iron concentration extracted from chrysotile-gypsum waste over 24 h in
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5 supernatants (SN). Error bars indicate the standard errors of the means of five replicates.

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7 **In color online**

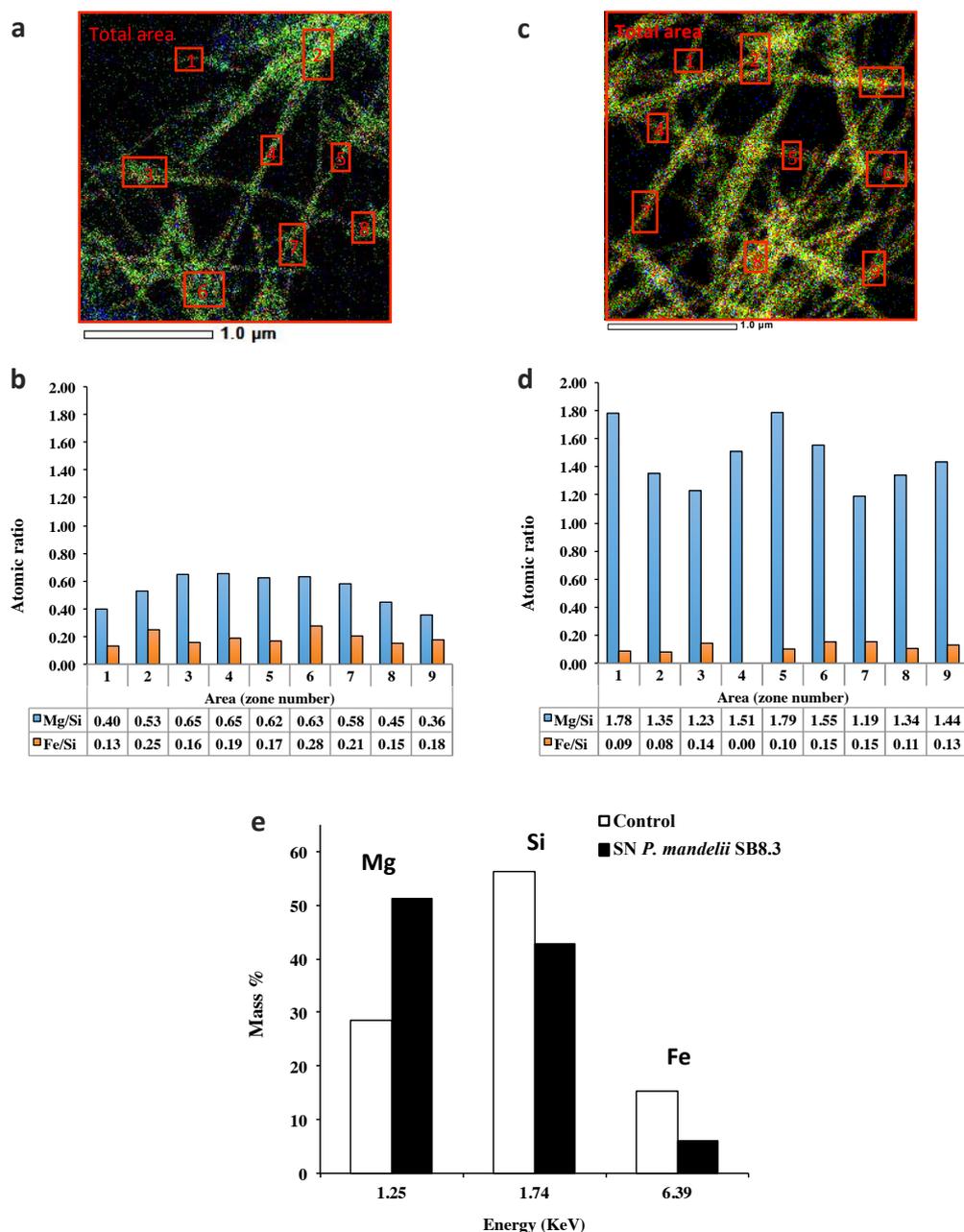
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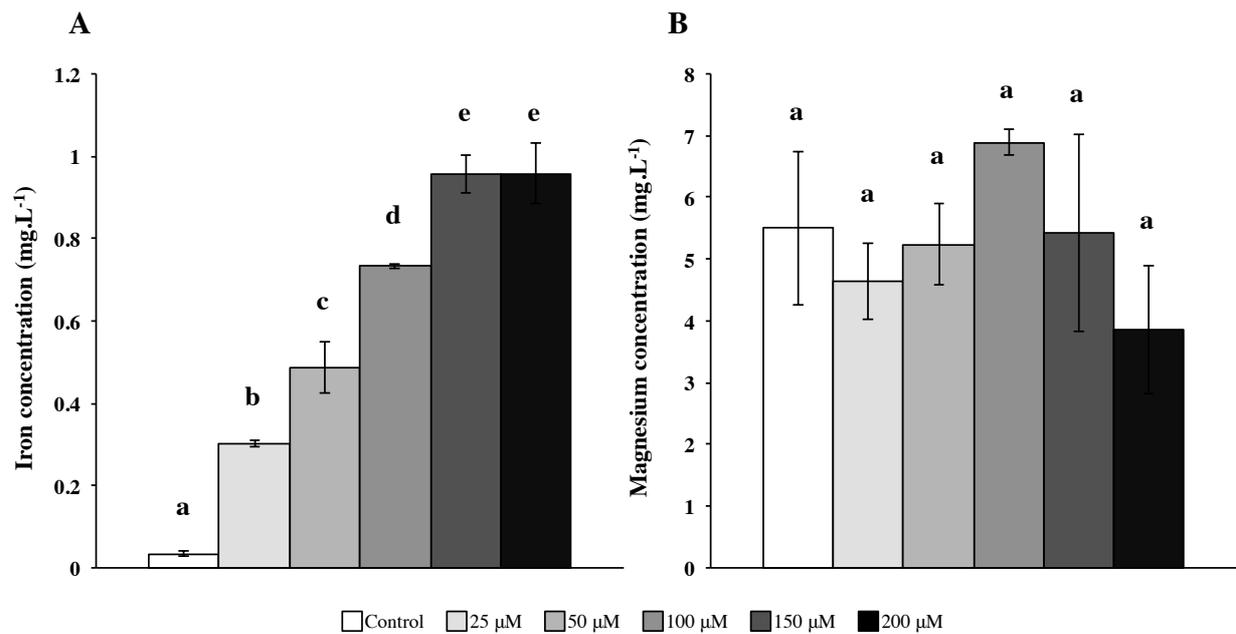
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2
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7 ratios of Mg/Si and Fe/Si (b and d). (e) Comparison histogram after renewal cycles with
8 succinate medium or *Pseudomonas mandelii* pyoverdine-containing supernatants.

9
10 **In color online**



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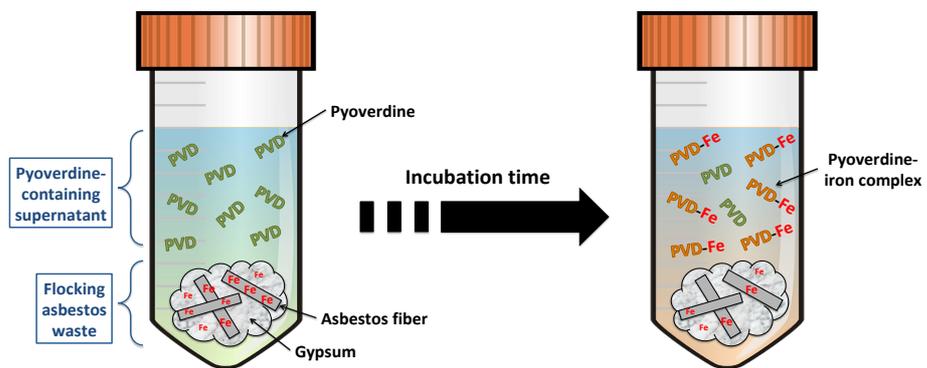
2

3 **Fig. 5.** Effect of various concentrations of pyoverdine produced by *Pseudomonas mandelii* on
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6 letter are not significantly different ($p > 0.05$, Kruskal-Wallis test of five replicates).

7

1 Graphical Abstract

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